



Methylated DNA

Instructions For Use

FOR RESEARCH USE ONLY (RUO).
NOT FOR USE IN DIAGNOSTIC
PROCEDURES.



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QCS17100



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Intended Use

FOR RESEARCH USE ONLY (RUO).

The result shall not be used for clinical diagnosis nor patient management. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.

The **Methylated DNA** can be used as external sample control to test if bisulfite conversion of DNA is successful. The **Methylated DNA** is a mixture of Human background DNA with DNA of a human methylated cell line. This results in DNA with significant methylation levels. The **Methylated DNA** can be used with the Self-screen Methylation specific PCR kits (PreCursor-M+, QIASure Methylation Test, PreCursor-M AnOYN and PreCursor-M Gold) but also with other human DNA methylation detection kits.

Summary and explanation

DNA methylation is a biochemical process that is important for normal development in higher organisms. It involves the addition of a methyl group to the 5th position of the pyrimidine ring of the cytosine nucleotide. Abnormal patterns of DNA methylation also play a role in carcinogenesis and several assays have been developed to test the methylation level of certain genes that are seemingly involved in carcinogenesis. The **Methylated DNA** can be used as external sample control to test the complete workflow for DNA methylation detection, including bisulfite conversion and detection by Methylation specific PCR kits. When certainty about experimental results is needed, the **Methylated DNA** sample can be included in each experiment. Alternatively, the frequency of adding the **Methylated DNA** sample in an experiment may be included with a certain frequency to collect monitoring data, or to just use it in case of trouble shooting.

Materials provided

Methylated DNA (10 ng/μl)	Two tubes of 100 μl
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Materials required but not provided

Consumables and reagents for bisulfite-conversion

Compatible bisulfite-conversion kits:

- EZ DNA Methylation Kit™ (ZYMO Research, cat.no. D5001 or cat.no. D5002)
- EZ DNA Methylation Lightning™ (ZYMO Research, cat.no. D5030 or cat.no. D5031)

The **Methylated DNA** can be used with any bisulfite conversion kit. Depending on the kit, DNA might need to be concentrated or diluted to obtain a suitable input concentration. Consult the instruction for use of the specific bisulfite conversion kit to find the suitable DNA concentration.

Methylation specific PCR assays

The **Methylated DNA** can be used in combination with any methylation specific detection assay, among which are the following:

Assay	Catalogue number	Distributed by
QIASure Methylation Test	616014	QIAGEN
QIASure Methylation Test-RUO	616015	QIAGEN
PreCursor-M Gold (RUO)	QHR14100	Self-screen BV and Fujirebio
PreCursor-M+	QHR15100	Self-screen BV and Fujirebio
PreCursor-M AnoGYN (RUO)	QHR16100	Self-screen BV and Fujirebio

For consumables, reagents and equipment for bisulfite conversion and the real-time methylation-specific PCR check the handbook of the manufacturer.

Equipment for handling **Methylated DNA**

- Adjustable pipets (1–10 µl; 10–100 µl) and filter tips
- Disposable gloves
- Benchtop centrifuge
- Vortex mixer

Warnings and precautions

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Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDS).

General precautions

Use of DNA samples and PCR assays requires good laboratory practices, including maintenance of equipment, that are dedicated to molecular biology and is compliant with applicable regulations and relevant standards. Always pay attention to the following:

- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Prevent microbial and nuclease (DNase) contamination of the specimen and the kit. DNase may cause degradation of the DNA template.
- Sample preparation and PCR set up must be performed in dedicated area separated from the area in which PCR amplification will be performed. To minimize the risk for PCR reaction contamination the use of a PCR-cabinet with UV-radiation is strongly recommended.
- The working area needs to be kept clean especially from PCR contaminants. Cleaning can

be performed with 0.1 N HCl and/or UV-radiation, or by a user preferred and proven method.

- Include a no template control in your PCR experiments to confirm that reagents are free of contamination.
- Avoid DNA or PCR product carryover contamination, which could result in a false-positive signal.

Reagent storage and handling

Methylated DNA is shipped on dry ice. Upon receipt store immediately at -30 to -15°C in a constant temperature freezer. The kit must be stored in a laboratory that is free of PCR amplicons. When stored under the specified storage conditions, Methylated DNA is stable until the expiration date stated on the box label. Once opened, reagents can be stored in their original packaging at -30 to -15°C.

Repeated thawing and freezing should be avoided, the tubes contain check boxes that can be used to indicate the amount of freeze-thaw cycles. Do not exceed a maximum of 5 freeze-thaw cycles. Before use, thaw the reagents by placing them at room temperature for 15 minutes. Mix by gently vortexing and centrifuge the tube before opening.

Procedure

- Take a tube of **Methylated DNA** from the freezer and allow the sample to thaw for 15 min at room temperature.
- Mix by gently vortexing and briefly centrifuge the tube before opening.
- Take 20 µl of DNA from the tube to be used with the EZ DNA Methylation Kit™ or the EZ DNA Methylation Lightning kit (ZYMO Research). When using another bisulfite conversion kit of choice DNA might need to be concentrated or diluted to obtain a suitable DNA input concentration.
- Mark a check box on the tube when it has been thawed, and put it back in the freezer.
- Follow the manufacturers protocol for bisulfite conversion.
- Perform a PCR with a methylation specific PCR assay of choice according to the manufacturers protocol.

Expected results

The **Methylated DNA** has been tested with the assays indicated in table below, for these assays it is indicated what range of Ct values can be expected. When results fall (consistently) outside of this range it is advised to start a root cause investigation on your complete workflow (including bisulfite conversion kit, PCR assay, used equipment, used pipettes, etc.).

Assay and PCR cyclor:	Precursor-M+ on Mic qPCR cyclor		
Marker:	ACTB	FAM19A4	hsa-mir124-2
Range for Ct value	22,18 - 25,18	28,17 - 31,17	30,35 - 33,35
Assay and PCR cyclor:	QIAsure Methylation test on Rotor-Gene Q 5-plex HRM		
Marker:	ACTB	FAM19A4	hsa-mir124-2
Range for Ct value	22,58 - 25,58	27,91 - 30,91	30,29 - 33,29
Assay and PCR cyclor:	Precursor-M Gold on Mic qPCR cyclor		
Marker:	ACTB	LHX8	ASCL1
Range for Ct value	21,87 - 24,87	27,81 - 30,81	26,62 - 29,62
Assay and PCR cyclor:	Precursor-M AnoGYN on Mic qPCR cyclor		
Marker:	ACTB	ZNF582	ASCL1
Range for Ct value	21,97 - 24,97	26,61 - 29,61	27,41 - 30,41















For other PCR kits than indicated in the table the user can define own acceptance criteria. This could be done by testing the sample 10 times with the workflow for bisulfite-conversion and methylation detection assay. Determine your own range, for instance by determining the average Ct value of each marker and set a range of the average Ct plus and minus 1.5 Ct. From then on one can monitor if results fall consistently within that range.

Limitations

- **Methylated DNA** is for Research Use Only.
- Do not use components beyond their expiration date.
- Any off-label use of this product and/or modification of the components will void Self-screen B.V.'s liability.

Symbols

The following symbols may appear on the packaging and labelling:

Symbol	Symbol definition
	Use by
	Contains reagents sufficient for <N> reactions
	Catalogue number
	Lot number
	Material number
	Components
	Contains
	Number
Rn	R is for revision of the Instructions for Use (Handbook) and n is the revision number
	Global Trade Item Number
	Temperature limitation
	Manufacturer
	Protect from light
	Consult instructions for use
	Caution

Contact information

For technical assistance and more information, please contact Self-screen or our local distributors.

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Revision History

Revision	Changes
1	First version